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Intracellular Voltammetry at  
Ultrasmall Platinum Electrodes

by

Y. Y. Lau, D. K. Y. Wong, G. Luo, A. G. Ewing

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## ABSTRACT

Ultrasmall platinum ring electrodes have been constructed by electrodeposition of platinum onto carbon ring electrodes. Electrodes thus formed show similar adsorption/desorption peaks for hydrogen and oxygen in 0.50 M  $\text{H}_2\text{SO}_4$  solution compared to those obtained at conventional-sized electrodes. These electrodes have been used in conjunction with a technique involving linear scan voltammetry and subsequent cleaning pulses. In this technique, the applied potential is ramped anodically from an initial potential to a final potential, followed by a large positive potential to oxidatively clean the electrode and a large negative potential for electrode activation. Intracellular measurements of dopamine at platinum ring electrodes have then been studied by integrated pulse linear scan voltammetry. In all experiments, minimal electrode fouling allows repeatable quantitative measurements to be carried out.

## INTRODUCTION

A number of microelectrodes of different geometries have now been reported in the literature [1, 2]. These include microdisk [3, 4] and arrays of microdisks [5-8], microband [9-13], microcylinder [14, 15], and ultrathin ring electrodes [16]. While many of these electrodes have very small areas, in general they have considerably larger total structural diameters, making them unsuitable for use in microenvironments (e.g. cytoplasm of single cells). Hence, it is highly desirable to construct electrodes with total structural diameters of only a few micrometers. Meulemans et al. [17, 18] have employed 0.5-2  $\mu\text{m}$  tip diameter carbon fiber and platinum electrodes to the intracellular measurement of endogenous ascorbic acid and serotonin in single cells. In our laboratory, carbon ring electrodes of 1-5  $\mu\text{m}$  structural diameters [19, 20] have been fabricated by pyrolysis of methane on the inside wall of pulled quartz capillaries which are then sealed with epoxy. The small size of carbon ring electrodes has enabled voltammetric measurements to be made in the cytoplasm of the large dopamine neuron of the pond snail *Planorbis corneus* [21-23]. Most of the above experiments have been carried out with carbon electrodes. However, the use of other electrode substrates, e.g. platinum and gold electrodes, is expected to expand our repertoire of surfaces for structurally small electrodes.

Recently, Pendley and Abruna [24] reported the construction of submicron platinum disk electrodes by pulling glass pipets to encase a very fine platinum wire. Also, Penner et al. [25] have

been successful in the fabrication of nanometer-sized platinum and platinum-iridium electrodes. However, noble metal electrodes are generally not used in biological experiments. This is probably due to the relative ease of fouling of these electrodes in biological media. Strong adsorption, for example, that of high molecular weight species in the cell cytoplasm (e.g. proteins, lipids, sugars) on the electrode surface, usually results in a reduction of the amperometric signal. Thus, continuous detection of sample species, either qualitatively or quantitatively, becomes very difficult in biological experiments.

Noble metal electrodes have been employed for HPLC detection by Johnson and LaCourse using multiple pulse techniques [26]. Many aliphatic alcohols and amines are predicted to be easily oxidized based on thermodynamic data, but undergo oxidation at very slow reaction rates and are often considered nonelectroactive in the potential range obtained in aqueous solutions. Johnson and LaCourse [26] have pointed out that this results from the absence of  $\pi$ -resonance by aliphatic free-radical products in electro-oxidation reactions. Adsorption and stabilization of these free-radical products can occur when the activation barrier for oxidation of aliphatic compounds is decreased at noble metal electrodes (e.g. Pt and Au) with partially unsaturated surface d-orbitals. However, the same properties that lead to strong adsorption of these solutes on electrodes also lead to fouling by the accumulated reaction products.

In order to minimize the degree of electrode fouling while

maintaining the reactivity of platinum and gold electrodes, Johnson and coworkers [27, 28] have developed an integrated pulse amperometric detection technique (previously known as potential sweep-pulsed coulombic detection). In this technique, the applied potential waveform consists of a rapid cyclic potential sweep followed by large positive and negative potential pulses. The positive potential oxidatively cleans the electrode surface, and the negative potential dissolves the metal oxide formed during cleaning and restores the native reactivity of the clean metal surface. The result of these electrochemical pretreatment steps between successive potential scans is the ability to carry out continuous amperometric monitoring of many species ordinarily not determined by voltammetry. In integrated pulse amperometric detection, the coulombic charge, obtained by integrating the electrode current during the cyclic sweep, is then plotted for each complete scan.

In this paper, we report procedures to construct and characterize structurally small platinum ring electrodes. These electrodes are employed in conjunction with a potential waveform consisting of a linear anodic potential scan, followed by a large positive and a large negative potential pulses. This technique is similar to that used by Johnson and coworkers for integrated pulse amperometric detection in HPLC. In this paper, we use the scan-pulse method to obtain voltammetric information at small platinum electrodes placed in single cell cytoplasm.

## MATERIALS AND METHODS

**Chemicals.** Dopamine was purchased from Sigma Chemical Co., and sulfuric acid, nitric acid, hydrochloric acid, citric acid, and sodium hydrogen phosphate heptahydrate were obtained from J. T. Baker. All solutions were made with doubly distilled water (Corning Mega-Pure MP-3A). Solutions of  $1.0 \times 10^{-3}$  M  $\text{H}_2\text{PtCl}_6$  were made by dissolving platinum powder (1.4  $\mu\text{m}$ , 99.9% purity, Johnson Matthey Inc.) in aqua regia. All solutions were purged with nitrogen for 20 min prior to carrying out experiments and a blanket of nitrogen was kept over the solutions at all times.

**Apparatus and Electrodes.** Cyclic voltammetry and integrated pulse linear scan voltammetry were carried out with an EI-400 potentiostat (Ensmann Instrumentation, Bloomington, IN). Potential waveforms for integrated pulse linear scan voltammetry and data acquisition were carried out with an IBM personal computer and a commercial interface (Labmaster, Scientific Solutions, Solon, OH). *In vitro* experiments employed an electrochemical cell consisting of a 30 ml glass vial, filled to 25 ml, with holes drilled in a plastic cap to facilitate a three-electrode system. A sodium chloride saturated calomel electrode (SSCE) was used as the reference electrode and a platinum wire as the auxiliary electrode. All experiments were carried out in a copper mesh Faraday cage at room temperature.

Ultrasmall carbon ring electrodes were constructed as described previously [19, 20, 29]. Carbon ring electrodes were

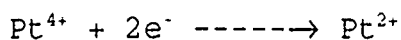


then placed in a solution of  $1.0 \times 10^{-3}$  M  $\text{H}_2\text{PtCl}_6$  and 0.50 M  $\text{H}_2\text{SO}_4$  and reduction of platinum was carried out potentiostatically at 0.0 V vs SSCE. The platinum electrodes thus formed were rinsed thoroughly with doubly distilled water before being transferred into other solutions. Electrochemical reversibility was characterized by the "waveslope", which was calculated for each voltammogram by plotting  $-\log\{(i_{1,lm}-i)/i\}$  vs potential (E) to check for deviation from Nernstian behavior.

***In vivo* Preparation and Intracellular Measurements.** Intracellular voltammetry was carried out as described previously [22] except the integrated pulsed linear scan voltammetry technique was used. *In vitro* pre- and post-calibration plots were obtained prior to and after intracellular measurements, respectively. Errors are reported as standard error of the mean (SEM).

## RESULTS AND DISCUSSION

**Characterization of Platinum Electrodes.** Platinum ring electrodes have been constructed by electrodepositing platinum onto carbon ring electrodes. Georgolios et al. [30] have described this electrodeposition process as an irreversible reaction, corresponding to the successive reduction of  $\text{Pt}^{4+}$ :



In order to ensure that electrolysis does indeed result in platinum deposition on the electrode surface the following

experiments have been carried out. Figure 1(a) shows a background voltammogram obtained in 0.5 M  $\text{H}_2\text{SO}_4$  at a 3  $\mu\text{m}$  total tip diameter carbon ring electrode following platinum deposition for 40 s. The general features of the voltammogram are comparable to those obtained at larger diameter ultrathin platinum ring electrodes [16] and at platinized carbon fibers [30]. All peaks associated with adsorption/desorption of hydrogen and oxygen, respectively, are present. For comparison, a cyclic voltammogram obtained with a 5  $\mu\text{m}$  tip diameter carbon ring electrode in 0.50  $\text{H}_2\text{SO}_4$  is displayed in Figure 1(b). No corresponding adsorption/desorption peaks for hydrogen and oxygen are observed. Georgolios et al. [30] have speculated that electrochemically deposited platinum particles are attached to the carbon surface so firmly that rinsing will not dislodge them. In the experiments reported here, rinsing the electrode does not appear to affect the voltammetry obtained in  $\text{H}_2\text{SO}_4$  solution.

In another experiment, a thinner layer of platinum was deposited on a 4  $\mu\text{m}$  tip diameter carbon ring electrode, by use of a deposition duration of only 10 s. The voltammogram obtained in 0.50 M  $\text{H}_2\text{SO}_4$  is shown in Figure 1(c). In comparison to Figure 1(a), the general features of the voltammogram described previously are still observed, although the magnitude of the current for all adsorption/desorption peaks is diminished. Additionally, Figure 1(c) shows a lack of fine structure associated with the adsorption/desorption of hydrogen. Georgolios et al. [30] have observed that platinum deposits on carbon fiber bundles do not

display characteristic hydrogen adsorption peaks unless thick deposits have been formed (e.g. minimum of 0.3 mC passed for each mg of carbon fibers). Platinum ring electrodes can be used to obtain voltammetry of catecholamines. Cyclic voltammograms for the oxidation of dopamine in pH 7.4 citrate/phosphate obtained at platinum-coated ring electrodes show a well-defined sigmoidal response (Figure 2).

The small structural dimension of the electrodes employed here precludes morphological examination by conventional microscopy. It is difficult to determine the thickness of the carbon rings which, may be different even for electrodes of similar structural diameters. Thus, electrodeposition of platinum on carbon ring electrodes has been carried out potentiostatically for a desired duration in this work, rather than galvanostatically. With a thicker layer of platinum deposited after a longer reduction duration, cyclic voltammograms in  $\text{H}_2\text{SO}_4$  show all the adsorption/desorption peaks of hydrogen and oxygen, but cyclic voltammograms in  $1.0 \times 10^{-4}$  M dopamine show a large capacitive charging current. In contrast, a shorter reduction duration yields electrodes which do not show well-defined adsorption/desorption peaks of hydrogen and oxygen; however, a sigmoidal response with a small charging current is obtained for the oxidation of dopamine. Hence, for the work discussed in the remaining part of this paper, platinum was electrodeposited onto carbon ring electrodes for durations ranging from 10 s to 6 min. No effort has been made to correlate the charge passed during metal deposition to the amount

of metal deposited since the fraction of metal that is deposited on the thin carbon ring is unclear. This is especially true at microelectrodes where mass transport is enhanced relative to electrode area [1] and this is apparent in reports of electrodeposition of polymers on microelectrodes [31].

**Description of Integrated Pulse Linear Scan Voltammetry.** Pulsed amperometric detection (PAD) [26-28] has been widely used to maintain the reactivity of noble metal electrodes in HPLC. The use of the PAD concept combined with voltammetry should provide some selectivity in monitoring biological species *in vivo*. We have recently combined the PAD concept with pulse voltammetry at small platinum electrode for *in vivo* measurements [32]. The voltammograms obtained provide voltammetric information while maintaining a clean electrode surface. However, the multiple pulse waveform is relatively slow (approx. 20 s), hence a faster waveform is desirable. This can be accomplished by use of linear scan voltammetry at relatively high potential scan rates.

The waveform for integrated pulse linear scan voltammetry is shown in Figure 3. A rapid linear scan voltammogram is followed by a large anodic potential ( $E_a$ ) for oxidative cleaning of the electrode surface and subsequently by a cathodic potential ( $E_c$ ) for reactivation by dissolution of surface oxide. Voltammograms obtained at ultrasmall platinum ring electrodes using this waveform show a well-defined sigmoidal response for the oxidation of dopamine. Cleaning potential pulses after each scan maintain the

reactivity of the electrode surface and thus provide more sensitive and reproducible measurements which cannot be achieved by voltammetry alone.

**Voltammetry in Single Cell Cytoplasm.** An understanding of cellular neurochemistry and cell body involvement in neurotransmission and neuromodulation should be greatly facilitated by measurements at the single cell level. The platinum ring electrodes described here have been used to carry out voltammetry in the cell body of the dopamine neurons of *Planorbis corneus*. The average ratio of two current values from pre- and post-calibration plots, selected in the lower and higher concentration range, respectively, has been used to calculate the fraction of the electrode response remaining after the intracellular experiment. An average loss of response of  $36 \pm 0.6\%$  [standard error of the mean (SEM);  $n = 5$ ] has been obtained for these measurements and this value is consistent with previous work using multiple pulse voltammetry at platinum electrodes [32].

Figure 4 shows the oxidation current monitored at +0.8 V for repeated linear scan voltammograms obtained intracellularly following repeated bathing of the cell with 0.5 mM dopamine. The increase in oxidation current for each peak apparently represents active transport of dopamine across the cell membrane. The rapid decay represents dopamine metabolism and/or vesicularization in the cell.

In Figure 4, with the exception of the first peak, reproducible peak responses to dopamine have been obtained, indicating an equal amount of dopamine being transported across the

membrane following each bathing. The average peak concentration as determined from the post-calibration is  $44 \pm 2 \text{ } \mu\text{M}$  (SEM,  $n = 22$ ). The rate of dopamine vesicularization and/or metabolism (clearance of dopamine from cytoplasm) has been estimated by examining the linear portion of each response during neurotransmitter clearance from the cytoplasm (declining limiting current). These results indicate a reproducible average combined rate of clearance of dopamine within the neuron of *Planorbis corneus* to be  $0.29 \pm 0.03 \text{ } \mu\text{M/s}$  (SEM,  $n = 22$ ). It should be noted that the current at the peak (top point) of each response is not used for these estimates. The different rate of clearance observed in the first seven seconds (one voltammogram) after the peak of the response might represent a two-phase clearance mechanism. Rapid scan rate voltammetry and data collection at higher frequency will be necessary to resolve this issue and will be the subject of future work.

Figure 5 shows two baseline-corrected voltammograms taken from the first and the last peaks of Figure 4. The limiting current of the voltammogram from the first peak is larger than that from the last and the waveslopes are 183 and 206 mV/decade, respectively. When we compare the above waveslopes to those for pre- and post-calibrations (171 and 207 mV/decade, respectively), the waveslopes of the voltammograms of the first and the last peaks are similar to the pre- and post-calibration, respectively. These results indicate the electrode response at the later stage of the cytoplasmic experiment is similar to that of the post-calibration with some electrode fouling occurring during the initial

voltammetric scans. It is also supported by the calculated average loss of response above, giving rise to a somewhat smaller response in later peaks. Despite the initial fouling of the electrodes, integrated pulse linear scan voltammetry has generally enabled repeatable voltammetric measurements.

### CONCLUSIONS

Use of platinum electrodes inside the neuron of *Planorbis corneus* has been demonstrated by integrated pulse linear scan voltammetry. The procedure reported in this work represents a simple methodology for constructing ultrasmall platinum ring electrodes. Integrated pulse linear scan voltammetry has been shown to reduce the percentage of electrode fouling by only 36% after 20 min of continuous voltammetry inside a single nerve cell and leads to more reliable quantitative analysis. Repeatable quantitative measurements of the amount of dopamine transported across the membrane of the neuron of *Planorbis corneus*, and the rate of vesicularization and/or metabolism of dopamine within the neuron are found to be 44  $\mu\text{M}$  and 0.29  $\mu\text{M/s}$ , respectively.

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### FIGURE CAPTIONS

Figure 1 (a) Cyclic voltammogram obtained at a 3  $\mu\text{m}$  tip platinum-coated ring electrode in 0.50 M  $\text{H}_2\text{SO}_4$ . Platinum was electrodeposited from  $\text{H}_2\text{PtCl}_6$  for 40 s. Scan rate = 200  $\text{mV s}^{-1}$ . Peak predictions:  $\text{H}_c$ , ion adsorption of hydrogen;  $\text{H}_a$ , oxidation of adsorbed hydrogen;  $\text{O}_c$ , reduction of oxide layer;  $\text{O}_a$ , formation of adsorbed oxygen or layer of platinum oxide. Oxidation current is plotted down and to the left.

(b) Cyclic voltammogram obtained at a 5  $\mu\text{m}$  tip carbon ring electrode in 0.50 M  $\text{H}_2\text{SO}_4$ . Scan rate = 200  $\text{mV s}^{-1}$ . Oxidation current is plotted down and to the left.

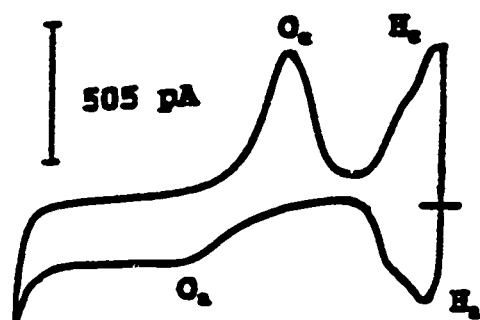
(c) Cyclic voltammogram obtained at a 4  $\mu\text{m}$  tip platinum-coated ring electrode in 0.5 M  $\text{H}_2\text{SO}_4$ . Platinum was electrodeposited for 10s. Scan rate = 200  $\text{mV s}^{-1}$ . Oxidation current is plotted down and to the left.

Figure 2 Cyclic voltammogram obtained at a 2  $\mu\text{m}$  tip platinum-coated electrode in  $1.0 \times 10^{-4}$  M dopamine (pH 7.4 citrate/phosphate buffer). Scan rate = 100  $\text{mV s}^{-1}$ . Oxidation current is plotted up and to the right.

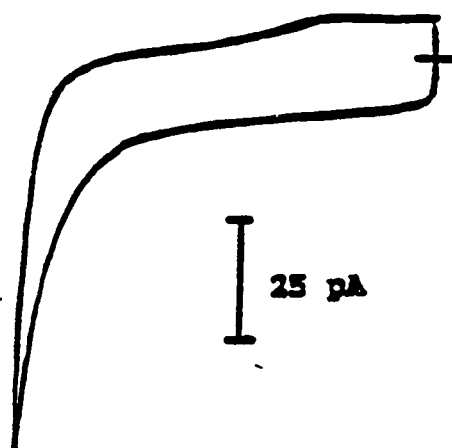
Figure 3 Schematic illustrating the waveform employed for integrated pulse linear scan voltammetry. The potential is scanned from  $E_i$  to  $E_f$ .  $E_a$  denotes an oxidative cleaning potential held for a time  $t_a$ ,  $E_c$  an electrode reactivation potential held for  $t_c$ .

Figure 4 Plot of oxidation current vs time for intracellular monitoring by the integrated pulse linear scan voltammetry method in the identified dopamine cell of *Plonorbis corneus*. The current measured at 0.8 V vs SSCE from each linear scan voltammogram is plotted and the cell was bathed with 50  $\mu$ l of 0.5 mM dopamine at the arrows. Voltammograms were obtained every 7 s.

Figure 5 Background subtracted voltammograms from the peaks of the first (a) and fourth (b) dopamine bath shown in Figure 4. Oxidation current is plotted up and to the right.



a

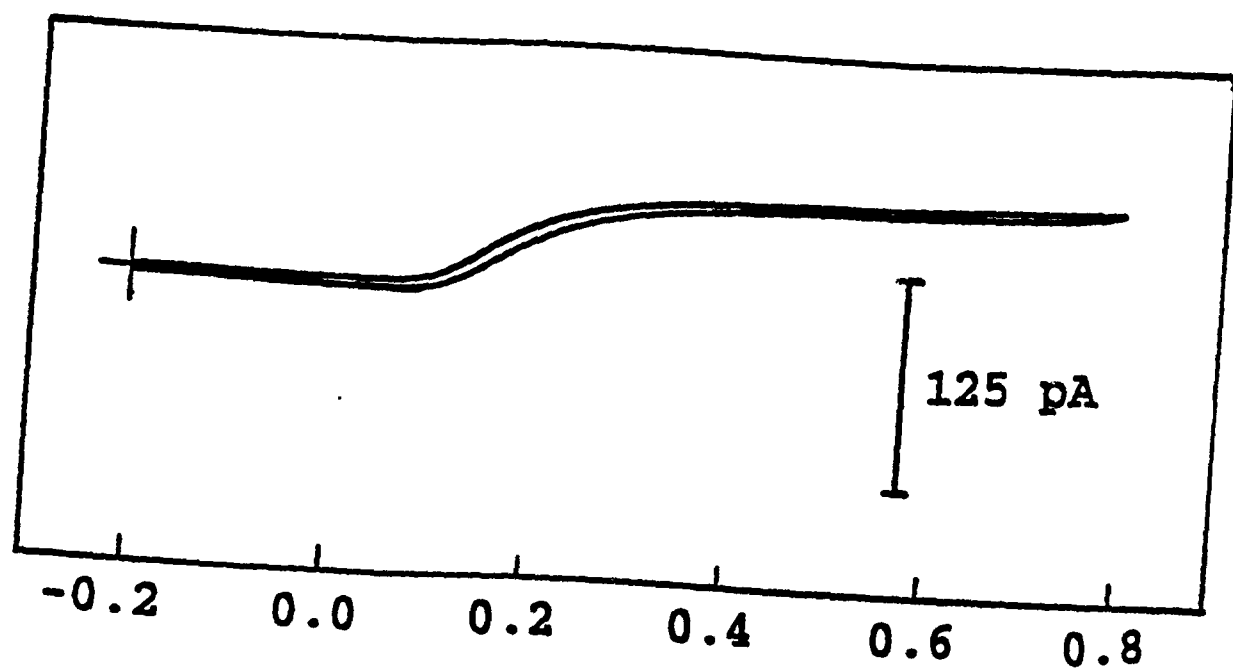


b



c

1.5 1.0 0.5 0.0 -0.5



$E$  vs SSCE (V)

